WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, October 28, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	(clostridium or clostridial or perfringens or beta).clm. same (promoter or transcript$ or secret$ or signal).clm.	3151
☐	L2	L1 and (nucleic or nucleotide or dna or cdna or mrna or m-rna or c-dna or polynucleotide or poly-nucleotide or nuclear or vector or cassette or transgene or trans-gene or heterologous or heter-ologous or stringent).clm.	1421
☐	L3	L2 and (transcript$).clm.	608
☐	L4	(clostridium or clostridial or perfringens).clm. near50 (promoter or transcript$ or secret$ or signal).clm.	32

END OF SEARCH HISTORY

Generate Collection | Print

Search Results - Record(s) 1 through 32 of 32 returned.

☐ 1. 20050142151. 11 Dec 03. 30 Jun 05. Immunogenic compositions including rough phenotype Brucella host strains and complementation DNA fragments. Nikolich, Mikeljon, et al. 424/252.1; A61K039/02 A61K039/10.

☐ 2. 20050019346. 27 May 04. 27 Jan 05. In vivo production of a clostridial neurotoxin light chain peptide. Boulis, Nicholas M.. 424/190.1; 435/252.3 435/320.1 435/69.3 530/350 536/23.7 C12P021/02 C07H021/04 C12N001/21 C07K014/33 C12N015/74.

☐ 3. 20050003357. 14 Jun 04. 06 Jan 05. Novel promoters inducible by dna damaging conditions or agents and uses thereof. Anne, Jozef, et al. 435/6; 435/199 435/320.1 435/325 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/22.

☐ 4. 20040197772. 30 Mar 01. 07 Oct 04. ISOLATION AND EXPRESSION OF A GENE FOR A NITRILASE FROM ACIDOVORAX FACILIS 72W. Chauhan, Sarita, et al. 435/6; 435/228 435/320.1 435/325 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/80.

☐ 5. 20040146963. 17 Mar 04. 29 Jul 04. High throughput assays for the proteotytic activities of clostridial neurotoxins. Schmidt, James J., et al. 435/23; 530/350 C07K014/33 A61K039/02 C12Q001/37.

☐ 6. 20040121347. 27 Feb 03. 24 Jun 04. Isolation and expression of a gene for a nitrilase from Acidovorax facilis 72W. Chauhan, Sraita, et al. 435/6; 435/227 435/320.1 435/325 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/78.

☐ 7. 20040071736. 05 Aug 03. 15 Apr 04. Methods and compounds for the treatment of mucus hypersecretion. Quinn, Conrad Padraig, et al. 424/239.1; 514/12 A61K039/08.

☐ 8. 20040058348. 18 Mar 03. 25 Mar 04. High level expression of heterologous proteins. Bogin, Oren, et al. 435/6; 435/189 435/252.3 435/320.1 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12P021/06 C12N009/02 C12N001/21 C12N015/74.

☐ 9. 20030165968. 27 Feb 03. 04 Sep 03. Isolation and expression of a gene for a nitrilase from Acidovorax facilis 72W. Chauhan, Sarita, et al. 435/6; 435/121 435/122 435/136 435/228 435/252.33 435/320.1 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12P017/10 C12P017/12 C12P007/40 C12N009/80 C12N001/21 C12N015/74.

☐ 10. 20030077685. 25 Sep 01. 24 Apr 03. High throughput assays for the proteotytic activities of clostridial neurotoxins. Schmidt, James J., et al. 435/23; 435/34 530/350 C12Q001/37 C12Q001/04 C07K014/33.

☐ 11. 20030059912. 27 Aug 02. 27 Mar 03. Hybrid protein for inhibiting the degranulation of mastocytes and the use thereof. Bigalke, Hans, et al. 435/188.5; 424/178.1 435/219 C12N009/50 C07K016/40 A61K039/40.

☐ 12. 20030049264. 20 May 02. 13 Mar 03. Clostridial toxin derivatives able to modify peripheral sensory afferent functions. Foster, Keith Alan, et al. 424/183.1; 424/247.1 435/69.1 435/69.3 435/69.7

530/350 A61K039/08 C12P021/06 C12P021/04 A61K039/40 A61K039/44 C07K014/00 C12N015/09 A61K039/395 A61K039/42 C07K001/00 C07K017/00.

☐ 13. 20020182229. 17 May 02. 05 Dec 02. Anaerobe targeted enzyme mediated prodrug therapy. Brown, John Martin, et al. 424/247.1; 435/320.1 A61K039/08 C12N015/74.

☐ 14. 20020107199. 17 Jan 02. 08 Aug 02. Methods of administering botulinum toxin. Walker, Patricia S.. 514/12; 514/44 A61K048/00 A61K038/16.

☐ 15. 20020068699. 23 Aug 01. 06 Jun 02. Clostridial toxin derivatives and methods for treating pain. Donovan, Stephen. 514/12; 530/350 A61K039/08 C07K014/33.

☐ 16. 20020022718. 19 Dec 00. 21 Feb 02. Genes identified as required for proliferation of E. coli. Forsyth, R. Allyn, et al. 536/23.1; 435/183 435/325 435/6 435/69.1 C07H021/02 C07H021/04 C12Q001/68 C12N009/00 C12P021/02 C12N005/06.

☐ 17. 6939548. 15 Aug 02; 06 Sep 05. Methods to produce high levels of C. difficile toxins. Wilkins; Tracy D., et al. 424/247.1; 424/184.1 424/185.1 424/192.1 424/200.1 424/234.1 424/9.1 424/9.2 530/300 530/350 536/23.7. A61K039/08 A61K049/00 A61K039/00 A61K039/02.

☐ 18. 6870038. 30 Mar 01; 22 Mar 05. Isolation and expression of a gene for a nitrilase from Acidovorax facilis 72W. Chauhan; Sarita, et al. 536/23.1; 435/320.1 435/6 536/24.3. C07H021/02 C07H021/04 C12Q001/68.

☐ 19. 6843998. 13 Apr 00; 18 Jan 05. Methods and compositions for the treatment of pancreatitis. Steward; Lance E., et al. 424/236.1; 424/197.11 424/198.1 424/247.1. A61K039/02.

☐ 20. 6822076. 27 Aug 02; 23 Nov 04. Hybrid protein for inhibiting the degranulation of mastocytes and the use thereof. Bigalke; Hans, et al. 530/350; 424/192.1 435/7.1 530/300. C07K001/00.

☐ 21. 6776990. 08 Apr 99; 17 Aug 04. Methods and compositions for the treatment of pancreatitis. Sachs; George, et al. 424/192.1; 424/193.1. A61K039/00.

☐ 22. 6746672. 19 Jun 01; 08 Jun 04. Isolated bifidobacteria that produce siderophores which inhibit growth of lactococcus lactis. O'Sullivan; Daniel J.. 424/93.4; 435/252.1 435/822. A01N063/00 C12N001/00 C12N001/12 C12N001/20.

☐ 23. 6632440. 29 May 01; 14 Oct 03. Methods and compounds for the treatment of mucus hypersecretion. Quinn; Conrad Padraig, et al. 424/239.1; 424/236.1 424/282.1 424/434 424/810 435/325 435/368 435/371 435/6 435/69.1 435/7.1 514/12 514/14 514/2 530/350. A61K039/68 A61K039/00 C07K014/00.

☐ 24. 6521235. 09 Mar 01; 18 Feb 03. Alphavirus RNA replicon systems. Johnston; Robert E., et al. 424/199.1; 424/218.1 435/235.1 435/236 435/320.1. A61K039/12 C12N007/01 C12N015/86.

☐ 25. 6444437. 14 Jul 99; 03 Sep 02. Process for the production of nutritional products with microorganisms using sequential solid substrate and liquid fermentation. Sporleder; Robert A., et al. 435/42; 424/93.1 424/93.3 426/54 435/195 435/201 435/209. C12P039/00 A01N063/00.

☐ 26. 5955368. 06 Apr 98; 21 Sep 99. Expression system for clostridium species. Johnson; Eric A., et al. 435/488; 435/252.3 435/320.1 435/476 536/23.1 536/24.1. C12N001/21 C12N015/70 C12N015/74

C12N015/64.

☐ 27. 5759845. 31 Jan 96; 02 Jun 98. Secretion of clostridium cellulase by E. coli. Yu; Ida Kuo. 435/277; 435/267 435/274. C12S003/02 C12S003/04.

☐ 28. 5496725. 11 Aug 93; 05 Mar 96. Secretion of Clostridium cellulase by E. coli. Yu; Ida K.. 435/252.3; 435/209 435/252.33 435/254.11 435/320.1. C12N001/15 C12N001/21 C12N005/10 C12N009/42.

☐ 29. 5436239. 01 Apr 93; 25 Jul 95. Method of treating clostridium difficile colitis and cholera. Guerrant; Richard L., et al. 514/219; 514/342 514/420 514/468. A61K031/34 A61K031/405 A61K031/44 A61K031/55.

☐ 30. 5177017. 22 Mar 90; 05 Jan 93. Molecular cloning of the genes responsible for collagenase production from Clostridium histolyticum. Lin; Hun-Chi, et al. 435/252.33; 435/220 435/320.1 536/23.7. C12N015/57 C12N015/70 C12N015/31.

☐ 31. 4905761. 29 Jul 88; 06 Mar 90. Microbial enhanced oil recovery and compositions therefor. Bryant; Rebecca S.. 166/246; 435/252.4 435/42. E21B043/22.

☐ 32. 4820714. 02 May 86; 11 Apr 89. Use of phospholipase inhibitors in the treatment of Clostridium difficile diarrhea. Guerrant; Richard L., et al. 514/297; 514/547 514/867. A61K031/44 A61K031/225.

Generate Collection Print

Term	Documents
CLOSTRIDIUM	9126
CLOSTRIDIUMS	1
CLOSTRIDIA	1224
CLOSTRIDIAS	1
CLOSTRIDIAL	710
CLOSTRIDIALS	1
PERFRINGENS	3160
PERFRINGEN	8
PROMOTER	101375
PROMOTERS	80062
SIGNAL	1414979
((CLOSTRIDIUM OR CLOSTRIDIAL OR PERFRINGENS).CLM. NEAR50 (PROMOTER OR TRANSCRIPT$ OR SECRET$ OR SIGNAL).CLM.).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD.	32

There are more results than shown above. Click here to view the entire set.

Prev Page Next Page Go to Doc#

US 20020182229A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0182229 A1

Brown et al. (43) Pub. Date: Dec. 5, 2002

(54) ANAEROBE TARGETED ENZYME MEDIATED PRODRUG THERAPY

(75) Inventors: John Martin Brown, Redwood City, CA (US); Nigel P. Minton, Salisbury (GB); Amato Giaccia, Stanford, CA (US)

Correspondence Address:
PERKINS COIE LLP
P.O. BOX 2168
MENLO PARK, CA 94026 (US)

(73) Assignee: The Board of Trustees of the Leland Stanford Junior University

(21) Appl. No.: 10/151,069

(22) Filed: May 17, 2002

Related U.S. Application Data

(63) Continuation of application No. 08/686,502, filed on Jul. 24, 1996, now Pat. No. 6,416,754, which is a continuation of application No. 08/465,932, filed on Jun. 6, 1995, now abandoned, which is a continuation of application No. 08/227,313, filed on Apr. 13, 1994, now abandoned, which is a continuation of application No. 08/206,430, filed on Mar. 3, 1994, now abandoned.

Publication Classification

(51) Int. Cl.[7] A61K 39/08; C12N 15/74

(52) U.S. Cl. 424/247.1; 435/320.1

(57) ABSTRACT

A genetically-engineered anaerobic organism is provided which, under anaerobic conditions present in a solid tumor, produces an enzyme capable of catalyzing the conversion of a prodrug to its highly cytotoxic product in situ and methods of treating tumors using same.

PGPUB-DOCUMENT-NUMBER: 20020182229
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020182229 A1

TITLE: Anaerobe targeted enzyme mediated prodrug therapy

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Brown, John Martin	Redwood City	CA	US
Minton, Nigel P.	Salisbury	CA	GB
Giaccia, Amato	Stanford		US

US-CL-CURRENT: 424/247.1; 435/320.1

CLAIMS:

We claim:

1. A vector expressed in obligate anaerobes for the production of an enzyme capable of converting a non toxic prodrug to a toxic chemotherapeutic agent.

2. The vector of claim 1, wherein the anaerobe is a member of the genus Clostridium.

3. The vector of claim 1, wherein the enzyme is nitroreductase.

4. The vector of claim 3, wherein the enzyme is nitroreductase and the anaerobe is a member of the genus Clostridium.

5. The vector of claim 4, wherein the anaerobe is Clostridium acetobutylicum.

6. The vector of claim 5, wherein the vector is an anaerobe vector comprising the ntr gene encoding E. coli B nitroreductase (NTR) and the promoter and RBS of the ferredoxin (Fd) gene of Clostridium pasteurianum.

7. The vector of claim 1, wherein the enzyme is .beta.-glucuronidase.

8. The vector of claim 7, wherein the anaerobe is a member of the genus Clostridium.

9. The vector of claim 8, wherein the anaerobe is Clostridium acetobutylicum.

10. The vector of claim 1, wherein the enzyme is cytosine deaminase.

11. The vector of claim 10, wherein the anaerobe is a member of the genus Clostridium.

12. The vector of claim 11, wherein the anaerobe is Clostridium acetobutylicum.

13. The vector of claim 2, wherein the prodrug is CB1954.

14. The vector of claim 3, wherein the prodrug is selected from the group consisting of glucuronides of epirubicin, 5-fluorouracil, and 4-hydroxycyclophosphamide.

15. The vector of claim 4, wherein the prodrug is 5-fluorocytosine.

16. A method of targeting a toxic chemotherapeutic agent to a tumor in a tumor-bearing individual comprising the steps of: a) administering an effective amount of a genetically engineered anaerobic microorganism capable of proliferating and producing an enzyme in the hypoxic/necrotic environment of a tumor to said individual; and then b) systemically administering a prodrug which is converted at the site of the tumor to the toxic chemotherapeutic agent by the enzyme produced by the microorganism.

17. The method of claim 16, wherein the anaerobic microorganism is a member of the genus Clostridium.

18. The method of claim 17, wherein the anaerobic microorganism is Clostridium acetobutylicum.

19. The method of claim 16, wherein the enzyme is nitroreductase.

20. The method of claim 16, wherein the prodrug is CB1954.

21. The method of claim 16, wherein the anaerobic microorganism is Clostridium acetobutylicum, the enzyme is E. coil B nitroreductase (NTR) and the prodrug is CB1954.

22. The method of claim 16, wherein the enzyme is .beta.-glucuronidase.

23. The method of claim 16, wherein the prodrug is selected from the group consisting of glucuronides of epirubicin, 5-fluorouracil, and 4-hydroxycyclophosphamide.

24. The method of claim 16, wherein the anaerobic microorganism is Clostridium acetobutylicum, the enzyme is .beta.-glucuronidase, and the prodrug is selected from the group consisting of glucuronides of epirubicin, 5-fluorouracil, and 4-hydroxycyclophosphamide.

25. The method of claim 16, wherein the enzyme is cytosine deaminase.

26. The method of claim 16, wherein the prodrug is 5-fluorocytosine.

27. The method of claim 16, wherein the anaerobic microorganism is Clostridium acetobutylicum, the enzyme is cytosine deaminase, and the prodrug is 5-fluorocytosine.

US006939548B2

(12) **United States Patent**
Wilkins et al.

(10) **Patent No.: US 6,939,548 B2**
(45) **Date of Patent: Sep. 6, 2005**

(54) **METHODS TO PRODUCE HIGH LEVELS OF *C. DIFFICILE* TOXINS**

(75) Inventors: **Tracy D. Wilkins**, Riner, VA (US); **David M. Lyerly**, Radford, VA (US); **J. Scott Moncrief**, Christiansburg, VA (US); **Limin Zheng**, Blacksburg, VA (US); **Carol Phelps**, Floyd, VA (US)

(73) Assignee: **Techlab, Inc.**, Blacksburg, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 372 days.

(21) Appl. No.: **10/222,038**

(22) Filed: **Aug. 15, 2002**

(65) **Prior Publication Data**

US 2003/0129198 A1 Jul. 10, 2003

Related U.S. Application Data

(62) Division of application No. 09/545,773, filed on Apr. 10, 2000, now Pat. No. 6,733,760.
(60) Provisional application No. 60/190,111, filed on Mar. 20, 2000, provisional application No. 60/186,201, filed on Mar. 1, 2000, and provisional application No. 60/128,686, filed on Apr. 9, 1999.

(51) **Int. Cl.**[7] **A61K 39/08**; A61K 49/00; A61K 39/00; A61K 39/02

(52) **U.S. Cl.** **424/247.1**; 424/9.1; 424/9.2; 424/184.1; 424/185.1; 424/192.1; 424/200.1; 424/234.1; 530/300; 530/350; 536/23.7

(58) **Field of Search** 424/9.1, 9.2, 184.1, 424/185.1, 192.1, 200.1, 234.1, 247.1; 530/300, 350; 536/23.7

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,530,833	A	7/1985	Wilkins et al.	424/92
4,533,630	A	8/1985	Wilkins et al.	435/7.32
4,863,852	A	9/1989	Wilkins et al.	435/7
4,879,218	A	11/1989	Wilkins et al.	435/7
5,098,826	A	3/1992	Wilkins et al.	435/7.32
5,736,139	A	4/1998	Kink et al.	424/164.1
5,919,463	A	7/1999	Thomas et al.	424/239.1

FOREIGN PATENT DOCUMENTS

WO	WO 96/12802	5/1996
WO	WO 97/02836	1/1997

OTHER PUBLICATIONS

Barroso et al. "Nucleotide Sequence of *Clostridum difficile* Toxin B Gene" Nucl. Acids Res. 18:4004 (1990).

Dove et al. "Molecular Characterization of the *Clostridum difficile* Toxin A Gene" Infect. Immun. 58:480–488 (1990).

Eichel–Streiber et al. "*Clostridum difficile* Toxin A Carries a C–Terminal Repetitive Structure Homologous to the Carbohydrate Binding Region of Streptococcal Glycosyltransferases" Gene 96:107–113 (1992).

Faust et al. "The Enzymatic Domain of *Clostridium difficile* Toxin A is Located within Its N–Terminal Region" Biochem. Biophys. Res. Commun. 251:100–105 (1998).

Hofmann et al. "Localization of the Glucosyltransferase Activity of *Clostridium difficile* Toxin B to the N–Terminal Part of the Holotoxin" J. Biol. Chem. 272:11074–11078 (1997).

Just et al. "Glucosylation of Rho Proteins by *Clostridium difficile* Toxin B" Nature 375:500–503 (1995).

Just et al. "The Enterotoxin from *Clostridium difficile* (ToxA) Monoglucosylates the Rho Proteins" J. Biol. Chem. 270:13932–13939 (1995).

Krivan et al. "Cell Surface Binding Site for *Clostridium difficile* Enterotoxin: Evidence for a Glycoconjugate Containing the Sequence . . . " Infect. Immun. 53:573–581 (1986).

Lyerly et al. *Infections of the Gastrointestinal Tract* Chapter 5, pp. 867–891 (1995).

Lyerly et al. "Vaccination Against Lethal *Clostridium difficile* Enterocolitis with a Nontoxic Recombinant Peptide of Toxin A" Current Microbiology 21:29–32 (1990).

Makoff et al. "Expression of Tetanus Toxin Fragment C in *E. coli*: Its Purification and Potential Use as a Vaccine" Bio/Technology 7:1043–1046 (1989).

Makoff et al. "Expression of Tetanus Toxin Fragment C in *E. coli*: High Level Expression by Removing Rare Codons" Nucleic Acids Res. 17:10191–10202 (1989).

Moncrief et al. "Positive Regulation of *Clostridium difficile* Toxins" Infect. Immun. 65:1105–1108 (1997).

Tucker et al. "Toxin A of *Clostridium difficile* Binds to the Human Carbohydrate Antigens I, X and Y" Infect, Immun. 59:73–78 (1991).

Primary Examiner—Rodney P. Swartz
(74) *Attorney, Agent, or Firm*—Morrison & Foerster LLP

(57) **ABSTRACT**

The present invention relates to the field of medical immunology and further to pharmaceutical compositions, methods of making and methods of use of vaccines. More specifically this invention relates to recombinant proteins derived from the genes encoding *Clostridium difficile* toxin A and toxin B, and their use in an active vaccine against *C. difficile*.

16 Claims, 11 Drawing Sheets

DOCUMENT-IDENTIFIER: US 6939548 B2
TITLE: Methods to produce high levels of C. difficile toxins

CLAIMS:

1. A method to produce the repeating unit portion of Clostridium difficile toxin A (rARU) or toxin B (rBRU) in high yield in E. coli bacteria which comprises culturing said bacteria under selective pressure, wherein said bacteria have been modified to contain a nucleic acid comprising an expression system which comprises a nucleotide sequence encoding said rARU or rBRU operably linked to an inducible promoter, whereby said rARU or rBRU is produced at levels of at least 10 mg/l of culture.

11. A method to produce the repeating unit portion of Clostridium difficile toxin A (rARU) in high yield in E. coli bacteria which comprises culturing said bacteria under selective pressure, wherein said bacteria have been modified to contain a nucleic acid comprising an expression system which comprises a nucleotide sequence encoding said rARU operably linked to an inducible promoter, whereby said rARU is produced at levels of at least 10 mg/l of culture.

US005955368A

United States Patent [19]

Johnson et al.

[11] Patent Number: 5,955,368

[45] Date of Patent: Sep. 21, 1999

[54] **EXPRESSION SYSTEM FOR CLOSTRIDIUM SPECIES**

[75] Inventors: **Eric A. Johnson**; **Marite Bradshaw**, both of Madison, Wis.; **Julian I. Rood**, Bentleigh; **Dena Lyras**, Heidelberg Heights, both of Australia

[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] Appl. No.: **09/056,075**

[22] Filed: **Apr. 6, 1998**

[51] **Int. Cl.**[6] **C12N 1/21**; C12N 15/70; C12N 15/74; C12N 15/64

[52] **U.S. Cl.** **435/488**; 435/320.1; 435/476; 435/252.3; 536/23.1; 536/24.1

[58] **Field of Search** 435/320.1, 172.3, 435/488, 476, 252.3; 536/23.1, 24.1

[56] **References Cited**

PUBLICATIONS

T.L. Bannam and J.I. Rood, "*Clostridium perfringens—Escherichia coli* Shuttle Vectors that Carry Single Antibiotic Resistance Determinants," *Plasmid* 229:223–235, 1993.

J.E. Brown and E.D. Williamson, "Molecular Approaches to Novel Vaccines for the Control of Clostridial Toxemias and Infections," *The Clostridia: Molecular Biology and Pathogenesis*, Academic Press, pp. 505–525, 1997.

B. Dupuy and A.L. Sonenshein, "Transcriptional Regulation of *Clostridium difficile* TOXA and TOXB Genes," p. 58, 1997 (Abstract).

E.A. Johnson and M. Bradshaw, "Genetic Characterization of Neurotoxin Complexes in *Clostridium botulinum* Type A," p. 39, 1997 (Abstract).

E.A. Johnson, et al., "Characterization of Neurotoxin Mutants in *Clostridium botulinum* Type A," *Clin. Infect. Dis.* 25(Suppl 2):S168–S170, 1997.

W.–J. Lin, "Characterization of Toxin—Negative Mutants of *Clostridium botulinum* 62A Produced by Transposon TN916 Mutagenesis," *The Development of Genetic Methods to Study Group I Clostridium botulinum* pp. 100–128, 1992.

W.–J. Lin and E.A. Johnson, "Genome Analysis of *Clostridium botulinum* Type A by Pulsed–Field gel Electrophoresis," *App. Env. Micro.* 61(12):4441–4447, 1995.

D. Lyras and J.I. Rood, "Conjugative Transfer of PR4–oriT Shuttle Vectors from *Escherichia coli* to *Clostridium perfringens*," *Plasmid* 39:160–164, 1998.

D. Lyras, et al., "Conjugative Transfer of Shuttle and Suicide Vectors From *Escherichia coli* to *Clostridium perfringens*," Second International Meeting on the Molecular Genetics and Pathogenesis of the Clostridia, pp. 10, 11 and 73, Jun. 22–Jun. 25, 1997 (Poster).

J. C. Marvaud, et al., "orf21 is a Positive Regulatory of Btulinum Neurotoxin and Associated Non–Toxic Protein Genes in C. botulinum,"p. 59, 1997 (Abstract).

N.P. Minton, "Molecular Genetics of Clostridial Neurotoxins," *Department of Molecular Biology*, Porton Down, Salisbury, UK, pp. 161–194, 19_.

E.J. Schantz and E.A. Johnson, "Properties and Use of Botulinum Toxin and Other Microbial Neurotoxins in Medicine," *Micro. Rev.* 56(1):80–99, 1992.

J.A. Schmidt, et al., "Development of a Reporter System to Study Neurotoxin Gene Expression in *Clostridium botulinum* Type A Strains," 1998.

J. Sloan, et al., "Construction of a Sequenced *Clostridium perfringens–Escherichia coli* Shuttle Plasmid," *Plasmid* 27:207–219, 1992.

T.C. Umland, et al., "The Crystal Structure of Tetanus Neurotoxin H_C Fragment," p. 38, 1997 (Abstract).

Primary Examiner—Johnny F. Railey, II

Attorney, Agent, or Firm—Quarles & Brady LLP

[57] **ABSTRACT**

A system is used to express clostridial gene constructions in a clostridial host. A mobilizable transfer plasmid is described which permits the direct transfer of the plasmid, and genes carried on it, from *E. coli* into Clostridium species. A promoter is described for use in clostridial species. Also, a useful host strain is used which is nontoxigenic and which permits high levels of expression of clostridial genes using the clostridial promoter.

15 Claims, 2 Drawing Sheets

DOCUMENT-IDENTIFIER: US 5955368 A
TITLE: Expression system for clostridium species

CLAIMS:

6. A method for the delivery and expression of genetic constructs in a Clostridium species comprising the steps of:

making a genetic construction including a promoter effective in the Clostridium species;

inserting the genetic construction in a mobilizable transfer plasmid which includes an origin of replication effective in E. coli; an origin of replication effective in a Clostridium species; a gene for an antibiotic resistance marker; and an origin of conjugative transfer which, when actuated, is capable of directing the transfer of the plasmid from E. coli into a Clostridium species;

transforming the mobilizable plasmid into an E. coli strain;

culturing the E. coli strain carrying the plasmid with a culture of the Clostridium species under conditions which facilitate conjugative transfer of the plasmid; and

selecting for bacteria of the Clostridium species which are hosting the plasmid.

8. A method as claimed in claim 6 wherein the promoter effective in Clostridium species is the NTNH promoter from Clostridium botulinum.

DOCUMENT-IDENTIFIER: US 5496725 A
TITLE: Secretion of Clostridium cellulase by E. coli

CLAIMS:

1. A recombinant microorganism comprising a vector for the heterologous expression of a cellulase enzyme wherein said enzyme is capable of digesting chemically or enzymatically untreated natural plant materials and said enzyme has a molecular weight of about 58,000 daltons as measured by gel exclusion chromatography wherein said microorganism comprises:

(a) an expression vector comprising a DNA sequence coding for said cellulase enzyme; and

(b) at least one DNA sequence coding for a signal sequence useful for the secretion of said cellulase enzyme, wherein a first DNA sequence of the expression vector codes for cellulase obtained from Clostridium strain IY-2.

3. A microorganism of claim 2 wherein said 2.8 kb insert is further cleaved into 2.2 kb DNA fragment comprising at least an entire structural gene and signal sequences of cellulase and the promoter is from the Clostridium cellulase gene on plasmid pPC1A.

US005496725A

United States Patent [19]

Yu

[11] Patent Number: 5,496,725

[45] Date of Patent: Mar. 5, 1996

[54] **SECRETION OF CLOSTRIDIUM CELLULASE BY *E. COLI***

[76] Inventor: **Ida K. Yu**, 3202 Monte Vista Pl., Davis, Calif. 95616

[21] Appl. No.: **105,870**

[22] Filed: **Aug. 11, 1993**

[51] **Int. Cl.**[6] **C12N 1/15**; C12N 1/21; C12N 5/10; C12N 9/42

[52] **U.S. Cl.** **435/252.3**; 435/209; 435/240.2; 435/252.33; 435/254.11; 435/320.1

[58] **Field of Search** 435/252.2, 252.8, 435/69.1, 842

[56] **References Cited**

PUBLICATIONS

Shima et al., J. Fermentation Bioeng. 68:75–78 (1989).
Gilkes et al., Biotechnology 2:259–262 (1984).
Ohmiya et al., Appl. Env. Microbiol. 55:2399–2402 (1989).
Fujino et al., J. Bacteriol. 171:4076–4079 (1989).
Yu, I. K. et al.; Abstr. Annu. Meet. Am. Soc. Microbiol., 87th Meeting, p. 272 (1987).
Shima, S.; Biol Abstr. 88(8): 84088 (1989).
Langsford, M. L. et al.; J. Gen. Microbiol. 130:1367–1376 (1984).

Primary Examiner—Robert A. Wax
Assistant Examiner—Eric Grimes
Attorney, Agent, or Firm—Albert P. Halluin; Pennie & Edmonds

[57] **ABSTRACT**

A gene, encoding an endocellulase from a newly isolated mesophilic Clostridium strain IY-2 which can digest bamboo fibers, cellulose, rice straw, and sawdust, was isolated by shotgun cloning in an *E. coli* expression plasmid pLC2833. *E. coli* positive clones were selected based on their ability to hydrolyze milled bamboo fibers and cellulose present in agar plates. One clone contained a 2.8 kb DNA fragment that was responsible for cellulase activity. Western blot analyses indicated that the positive clone produced a secreted cellulase with a mass of about 58,000 daltons that was identical in size to the subunit of one of the three major Clostridium cellulases. The products of cellulose digestion by this cloned cellulase were cellotetraose and soluble higher polymers. The cloned DNA contained signal sequences capable of directing the secretion of heterologous proteins from an *E. coli* host. The invention describes a bioprocess for the treatment of cellulosic plant materials to produce cellular growth substrates and fermentation end products suitable for production of liquid fuels, solvents, and acids.

5 Claims, 7 Drawing Sheets

DOCUMENT-IDENTIFIER: US 5177017 A
TITLE: Molecular cloning of the genes responsible for collagenase production from Clostridium histolyticum

CLAIMS:

4. The DNA fragment of claim 2 wherein the DNA derived from C. histolyticum contains a promoter within the inserted Clostridium DNA such that the DNA can be transcribed under control of the promoter to yield mRNA capable of being translated to yield the polypeptide of about 68,000 daltons without the functioning of a promoter external to the DNA derived from C. histolyticum.

6. A DNA fragment comprising the DNA fragment of claim 4 fused contiguously at least a portion of the structural gene for E. coli .beta.-galactosidase, the portion of the structural gene for .beta.-galactosidase being operatively linked to a lac promoter such that:

(i) the DNA derived from Clostridium can be transcribed under control of the promoter located within the Clostridium DNA to yield mRNA capable of being translated to yield the polypeptide of about 68,000 daltons without the functioning of a promoter external to the DNA derived from C. histolyticum; and

(ii) the Clostridium DNA fused to the DNA sequence comprising at least a portion of the structural gene for E. coli .beta.-galactosidase can be transcribed under control of the lac promoter to yield mRNA capable of being translated to yield a fusion polypeptide of molecular weight greater than 70,000 daltons displaying the antigenicity of C. histolyticum collagenase and containing at least a portion of the amino acid sequence of E. coli .beta.-galactosidase.

13. An isolated and purified recombinant DNA fragment comprising:

(a) a Clostridium histolyticum structural gene encoding C. histolyticum collagenase and capable of being transcribed to yield mRNA capable of being translated to yield polypeptides of molecular weight of from about 70,000 dalonts to about 100,000 daltons and having the enzymatic activity of C. histolyticum collagenase; and

(b) an internal promoter located within the structural gene of (a) and operatively linked to a Clostridium DNA sequence comprising a portion of the structural gene of (a) and capable of being transcribed to yield mRNA capable of being translated to yield a polypeptide of about 68,000 daltons molecular weight, the polypeptide having collagenase activity, the polypeptide being distinguishable by the heterologous expression of the sequence of claim 3 from the endogenous production by C. histolyticum of multiple forms of collagenase by the essential absence of those forms of C. histolyticum collagenease having molecular weights of above about 70,000 daltons determined by the expression of the C. histolyticum genomic coding sequence; the internal promoter being positioned such that the DNA sequence of (b) can be translated independently of the structural gene of (a).

US005177017A

United States Patent [19]

Lin et al.

[11] Patent Number: 5,177,017

[45] Date of Patent: Jan. 5, 1993

[54] **MOLECULAR CLONING OF THE GENES RESPONSIBLE FOR COLLAGENASE PRODUCTION FROM *CLOSTRIDIUM HISTOLYTICUM***

[75] Inventors: **Hun-Chi Lin; Shau-Ping Lei**, both of Los Angeles, Calif.

[73] Assignee: **Trigen, Inc.**, Santa Monica, Calif.

[21] Appl. No.: **498,919**

[22] Filed: **Mar. 22, 1990**

[51] Int. Cl.[5] **C12N 15/57**; C12N 15/70; C12N 15/31

[52] U.S. Cl. **435/252.33**; 435/220; 435/320.1; 935/14; 935/29; 935/73; 536/23.7

[58] Field of Search 435/69.1-69.9, 435/320.1, 220, 252.33; 536/27

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,705,083 12/1972 Chiulli et al. 435/70.2

OTHER PUBLICATIONS

Biochemistry, vol. 23, No. 22, issued Oct. 23, 1984, Sugasawara, et al., "Purification and Characterization of Three Forms of Collagenase From *Clostridium histolyticum*", pp. 5175-5181.

Proceedings of the National Academy of Sciences USA, vol. 82, May 1985, Young, et al., "Dissection of *Mycobacterium tuberculosis* Antigens Using Recombinant DNA", pp. 2583-2587.

Clinical Orthopedics and Related Research, No. 215, Feb. 1987, K. H. Chu, "Collagenase Chemonucleolysis Via Epidural Injection: A Review of 252 Cases", pp. 99-104.

Biochemistry, vol. 27, No. 19, Sep. 20, 1988, Angelton, et al., "Preparation and Reconstitution With Divalent Metal Ions of Class I and Class II *Clostridium histolyticum* Apocollagenases", pp. 7406-7412.

Biochemistry, vol. 27, No. 19, Sep. 20, 1988, Angleton et al., "Preparation by Direct Metal Exchange and Kinetic Study of Active Site Metal Substituted Class I and Class II *Clostridium histolyticum* Collagenases", pp. 7413-7418.

M. D. Bond & H. E. Van Wart, "Purification and Separation of Individual Collagenases of *Clostridium histolyticum* Using Red Dye Ligand Chromatography," *Biochemistry* 23, 3077-3085 (1984).

M. D. Bond & H. E. Van Wart, "Characterization of the Individual Collagenases from *Clostridium histolyticum*," *Biochemistry* 23, 3085-3091 (1984).

M. D. Bond & H. E. Van Wart, "Relationship Between the Individual Collagenases of *Clostridium histolyticum*: Evidence for Evolution by Gene Duplication," *Biochemistry* 23, 3092-3099 (1984).

M. F. French, K. A. Mookhtiar & H. E. Van Wart, "Limited Proteolysis of Type I Collagen and Hyperreactive Sites by Class I and II *Clostridium histolyticum* Collagenases: Complementary Digestion Patterns," *Biochemistry 26*, 681-687 (1987).

(List continued on next page.)

Primary Examiner—Robert A. Wax
Assistant Examiner—William W. Moore
Attorney, Agent, or Firm—Poms, Smith, Lande & Rose

[57] **ABSTRACT**

Genetically engineered *E. coli* carry vectors containing inserts that code for *Clostridium histolyticum* collagenase. These inserts code for: (a) a form of collagenase having a molecular weight of about 68,000 daltons in the essential absence of larger forms of collagenase; (b) the 68 kd form of collagenase and a fusion polypeptide consisting of the collagenase protein fused to at least a portion of the β-galactosidase protein of *E. coli;* or (3) the 68 kd form of collagenase and polypeptides of molecular weight of from above about 68,000 daltons to about 100,000 daltons and having the enzymatic activity of *C. histolyticum* collagenase as indicated by digestion of ^{3}H-acetylated collagen and by specific inhibition by 1,10-phenanthroline plus EDTA. The collagenase genes in the transformed *E. coli* are expressed efficiently in the transformed cells to yield enzymatically active and immunologically cross-reactive collagenase. In particular, the 68 kd form of collagenase is resistant to autocatalytic degradation and is stable to long-term storage. Genetically engineered collagenase, especially the 68 kd form that is resistant to autocatalytic degradation, can be used for isolation of pancreatic islets, for the isolation of dispersed tumor cells, or for treatment of "slipped disc."

23 Claims, 8 Drawing Sheets